Data Evaluation Record on the hydrolysis of benzyl diethyl

PMRA Submission Number {.....} EPA MRID Number 47135810

Data Requirement: PMRA Data Code:
EPA DP Barcode: D340822
OECD Data Point:
EPA Guideline: 161-1

Test material:

Common name:	Benzyl diethyl.
Chemical name:	
IUPAC name:	Cyclohexylmethyl-[2- [(2,6-dimethylphenyl)amino]- 2-oxoethyl]-diethylammonium benzoate.
CAS name:	Not reported.
CAS No.:	3734-33-6.
Synonyms:	Denatonium benzoate; Bitrex.
Smiles string:	c1ccccc1CN(CC)(CC)(OC(=O)c2ccccc2)CC(=O)Nc3c(C)cccc3C (EPI Suite, v3.12 SMILES).

Primary Reviewer: Kindra Bozicevich **Signature:**
Cambridge Environmental **Date:**

Secondary Reviewer: Kathleen Ferguson **Signature:**
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Final Reviewer: Jose Melendez **Signature:**
EPA Reviewer **Date:**

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 009106

CITATION: Hogg, A.S. and A.J. Barlett. 1995. Bitrex (denatomium benzoate NF grade 99.5% minimum) abiotic degradation, hydrolysis as a function of pH. Unpublished study performed by Safepharm Laboratories Ltd., Derby, DE1 2BT, United Kingdom; sponsored and submitted by Johnson Matthey Macfarlan Smith, Edinburgh, EH11 2QA, United Kingdom. Study Report No. SPL 799/007. Experiment started February 26, 1995 and completed date May 9, 1995 (p. 8). Final report issued September 21, 1995.

EXECUTIVE SUMMARY

The hydrolysis of non-radiolabeled cyclohexylmethyl-[2- [(2,6-dimethylphenyl)amino]- 2-oxoethyl]-diethylammonium benzoate (benzyl diethyl, Bitrex; purity 99.9%), at 203-206 mg a.i./L, was studied in sterile aqueous buffered pH 5 (0.1M phosphate), pH 7 (0.03M phosphate) and pH 9 (0.05M borate) solutions at 25 ± 0.5°C for 30 days. The study authors reported that the experiment was conducted in accordance with USEPA Guidelines for Pesticide Registration, Subdivision N §161-1 and in compliance with UK principles of GLP and meets the requirements of USEPA FIFRA GLP standards (40 CFR Part 160). It was not stated that experiment was conducted in the dark, or how darkness was maintained. The test system was not described; it appeared that a single bulk treated solution was prepared for each pH and these solutions were subsampled at each sampling interval. It was not reported whether the sample vessels were sealed; volatiles were not addressed. Aliquots of each test solution were collected for analysis at the following specified sampling intervals.

pH 5 and 9: *ca*. 0, 2.4, 120, 288, 479, and 723 hours posttreatment;
pH 7: *ca*. 0, 122, 312, 484, and 720 hours posttreatment.

Duplicate aliquots of the sample solution were diluted with methanol and analyzed using HPLC. Benzyl diethyl was identified by comparison to standards that were analyzed at each sampling interval. Transformation products were not addressed.

The test conditions presented in the study methods reportedly were maintained throughout the study; no supporting data were provided.

Mass balances were not determined.

Benzyl diethyl was stable in the pH 5, 7 and 9 buffer solutions, comprising 101%-104% of the applied at 720-723 hours posttreatment (*ca*. 30 days). Transformation products and volatiles were not addressed.

A transformation pathway could not be developed. Benzyl diethyl was stable to hydrolysis in all buffer solutions.

A supplementary experiment was conducted at 50°C in sterile aqueous buffered pH 4 (0.02M phosphate), pH 7 (0.03M phosphate), and pH 9 (0.05M borate) solutions. At 120 hours posttreatment (5 days), benzyl diethyl comprised 98.5%-101% of the applied. Transformation products and volatiles were not addressed.

RESULTS SYNOPSIS:

Treatment	Hal- life	Transformation products
pH 5, 25°C	Stable.	Transformation products were not addressed.
pH 7, 25°C	Stable.	Transformation products were not addressed.
pH 9, 25°C	Stable.	Transformation products were not addressed.

Study Acceptability: This study is classified as [to be filled in by the EFED reviewer]. It could not be determined if this experiment was conducted according to good scientific practices because the experimental methodology was not adequately described, so that the physical set-up was not known and it was not certain if the samples were kept in darkness. Also, a material balance could not be determined; quantitative data were provided only for benzyl diethyl. Transformation products were not quantified or identified, and volatiles were not addressed.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted according to Method C7 of Commission Directive 92/69/EEC, and USEPA Guideline §161-1 (p. 8). Significant deviations from the objectives of Subdivision N guidelines were noted:

> It could not be determined if this experiment was conducted according to good scientific practices. Important details of the experimental methodology were not reported, so the physical set-up of the samples was not known and it was not certain if the samples were kept in darkness.
>
> A material balance could not be determined; the study authors reported only data for benzyl diethyl. Transformation products and volatiles were not addressed.

COMPLIANCE: This study was conducted in compliance with the UK Principles of Good Laboratory Practice (1989), and reportedly meets the requirements of USEPA FIFRA GLP standards (40 CFR Part 160, pp. 3-5). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-6). A Certificate of Authenticity statement was not provided; however, the Quality Assurance Report included a statement that the study report was an accurate account of the data generated and the procedures followed (p. 6).

A. MATERIALS:

1. Test Material Benzyl diethyl (Bitrex; NF grade; p. 9).

Chemical Structure: See DER Attachment 1.
Description: White granular solid (p. 9).

Purity: Radiochemical purity: Not applicable, the test material was not radiolabeled.
Batch No.: 21119 (p. 9).
Analytical purity: 99.9%.
Specific activity: Not applicable.
Location of the radiolabel: Not applicable.

Storage conditions of test chemicals: Stored at room temperature (p. 9).

Physico-chemical properties of benzyl diethyl:

Parameter	Value	Comment
Molecular weight (g/mol)	446.59	
Molecular formula	$C_{28}H_{34}N_2O_3$.	
Water Solubility (mg/L)[1]	Not reported.	
Vapor Pressure/Volatility (Pa)	Not reported.	
UV Absorption	Not reported.	
pKa	Not reported.	
K_{ow}/log K_{ow}	Not reported.	
Stability of compound at room temperature, if provided	Not reported.	

1 At the website http://www.bitrex.com/pages/technical_data.htm, the solubility of Bitrex (benzyl diethyl) in water is reported to be 4.50 g/100 mL at 20°C.

2. Buffer Solution: Buffer solutions were prepared as follows:

Table 1: Description of buffer solutions.

pH	Type and molarity of buffer	Composition
5	0.1M Phosphate	0.10M Potassium dihydrogen orthophosphate solution added to 0.012M disodium hydrogen orthophosphate and adjusted with orthophosphoric acid.
7	0.03M Phosphate	0.028M Potassium dihydrogen orthophosphate solution added to 0.041M disodium hydrogen orthophosphate.
9	0.05M Borate	0.05M Disodium tetraborate solution added to 0.019M HCl.

Data obtained from p. 11 of the study report. No additional details were provided.

B. EXPERIMENTAL CONDITIONS

1. Preliminary Study: No preliminary studies were described.

2. Experimental conditions:

Table 2: Experimental parameters

Parameters		**Details**
Duration of study		30 days.
Test concentrations (mg a.i./L)	Nominal:	Not reported.
	Measured:	203-206 mg a.i./L ("concentration as weighed").
No. of replications		Not reported. Although duplicate aliquots of each treatment were analyzed at each interval, it is not known if these represent two samples or are a single divided sample.
Preparation of test medium	Volume used/treatment	Not reported.
	Method of sterilization	All buffer solutions were sterilized by filtration (0.22 μm) prior to use.
	Co-solvent	Not reported. The test substance may have been added to the buffer solutions as a solid.
Test apparatus		Not described.
Details of traps for volatile, if any		Volatile traps were not used.
If no traps were used, is the test system closed/open?		Not reported.
Is there any indication of the test material adsorbing to the walls of the test apparatus?		None.
Experimental conditions Temperature (°C): Lighting: pH ranges:		 25 ± 0.5°C. Not reported. Not reported.
Other details, if any		None.

Data were obtained from pp. 11-12, 23 of the study report.

3. Supplementary Experiments: A supplementary experiment was conducted at 50 ± 0.5°C in sterile aqueous buffered pH 4 (0.02M phosphate), pH 7 (0.03M phosphate), and pH 9 (0.05M borate) solutions treated with benzyl diethyl at 201-206 mg a.i./L (pp. 11-12, 19).

4. Sampling:

Table 3: Sampling details.

Criteria	Details
Sampling intervals	pH 5 and 9: *ca.* 0, 2.4, 120, 288, 479, and 723 hours posttreatment; pH 7: *ca*. 0, 122, 312, 484, and 720 hours posttreatment.
Sampling method	Not reported.
Method of collection of CO_2 and organic volatile compounds	Volatiles were not collected.
Sampling intervals/times for: pH measurement: Sterility check:	 Not determined. Not determined.
Sample storage before analysis	Not reported.
Other observation, if any:	None.

Data were obtained from pp. 12, . delete period 23 of the study report.

C. ANALYTICAL METHODS

Extraction/clean up/concentration methods: Duplicate aliquots from each pH buffer solution were diluted by a factor of 2 using methanol prior to HPLC analysis (p. 12).

Volatile residue determination: Volatiles were not trapped.

Total ^{14}C measurement: The test substance was not radiolabeled, and a material balance was not determined.. delete extra period

Derivatization method, if used: A derivatization method was not employed.

Identification and quantification of parent compound: Samples were analyzed by HPLC under the following operating conditions: Hichrom C_8/C_{18} RPB column (4.6 x 250 mm i.d.; particle size not reported), mobile phase of acetonitrile:5% acetic acid (25:75, v:v; adjusted to pH 3.5 using 1M NaOH), flow rate 1.0 mL/minute, with UV detection (254 nm; p. 13). Benzyl diethyl was identified by comparison to the behavior of reference standard solutions that were run in conjunction with each set of samples (p. 12; pp. 14-17).

Identification and quantification of transformation products: Transformation products were not addressed.

Detection limits (LOD, LOQ) for the parent compound: Limits of Detection and Quantitation were not reported.

Detection limits (LOD, LOQ) for the transformation products: Transformation products were not addressed.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: The test conditions presented in the study methods reportedly were maintained throughout the study; no supporting data were provided. It did not appear that the pH or sterility of the test solutions was confirmed at any interval.

B. MASS BALANCE: Mass balances were not determined.

Table 4a: Hydrolysis of benzyl diethyl, expressed as a percentage of the applied, in pH 4 and 9 buffer solutions at 25°C.

Compound	Sampling times (hours)					
	0	2.4	120	288	479	723
pH 5						
Benzyl diethyl	100	100	92.3	90.9	101	104
Transformation products	Not reported.					
Total volatiles	Not determined.					
Total Recovery	Not determined.					
pH 9						
Benzyl diethyl	100	100	100	99.0	99.0	101
Transformation products	Not reported.					
Total volatiles	Not determined.					
Total Recovery	Not determined.					

Data obtained from p. 23 of the study report. Only one value was provided for each sampling interval.

Table 4b: Hydrolysis of benzyl diethyl, expressed as a percentage of the applied, in a pH 7 buffer solution at 25°C.

Compound	Sampling times (hours)				
	0	122	312	484	720
pH 7					
Benzyl diethyl	100	103	105	105	101
Transformation products	Not reported.				
Total volatiles	Not determined.				
Total Recovery	Not determined.				

Data obtained from p. 23 of the study report. Only one value was provided for each sampling interval.

C. TRANSFORMATION OF PARENT COMPOUND: Benzyl diethyl was stable under the conditions of the study, comprising 104% of the applied in the pH 5 solution, 101% in the pH 7 solution, and 101% in the pH 9 solution at 720-723 hours posttreatment (*ca*. 30 days; p. 23).

HALF-LIVES/DT50/DT90: Benzyl diethyl was stable in buffer solutions at pH 5-9, with 30-day concentrations 101%-104% of the time 0 concentrations (p. 23). The study authors' estimated half-lives of >1 year (p. 24).

Half-lives/DT50/DT90

pH	First order linear			DT50[1]	DT90
	Half-life	Regression equation	r^2		
25°C					
5	Stable.			> 1 year	--
7	Stable.			> 1 year	--
9	Stable.			> 1 year	--

1 Estimated by the study authors (p. 24).

TRANSFORMATION PRODUCTS: Transformation products were not addressed.

VOLATILIZATION: Volatiles were not collected.

TRANSFORMATION PATHWAY: A transformation pathway could not be developed. Benzyl diethyl appeared stable to hydrolysis in all buffer solutions (p. 23).

Table 5: Chemical names and CAS numbers for the transformation products of benzyl diethyl.

Applicants Code Name	CAS Number	Chemical Name (IUPAC)	Chemical Formula	MW (g/mol)	Smiles String
Transformation products were not addressed.					

D. SUPPLEMENTARY EXPERIMENT-RESULTS: Benzyl diethyl appeared to be stable in pH 4, 7, and 9 buffer solutions incubated at 50°C (p. 19).

Table 6: Hydrolysis of benzyl diethyl, expressed as a percentage of the applied, in pH 4, 7 and 9 buffer solutions at 50°C.

Compound	Sampling times (hours)				
	0	2.4	24	75	120
pH 4					
Benzyl diethyl	100	97.6	99.0	100	98.5
Transformation products	Not reported.				
Total volatiles	Not determined.				
Total Recovery	Not determined.				
pH 7					
Benzyl diethyl	100	100	104	102	101
Transformation products	Not reported.				
Total volatiles	Not determined.				
Total Recovery	Not determined.				
pH 9					
Benzyl diethyl	100	99.5	99.5	99.0	101
Transformation products	Not reported.				
Total volatiles	Not determined.				
Total Recovery	Not determined.				

Data obtained from p. 19 of the study report.

III. STUDY DEFICIENCIES

1. Insufficient detail about the experimental methodology was provided, so it is not certain that good scientific practices were followed throughout the study. The physical set-up was not described, and it could not be determined if the test solutions were bulk solutions that were repeatedly subsampled or individual samples. It was not reported how (or if) the samples were kept in darkness. Other details of the incubation, such as whether a cosolvent was present, where the samples were incubated and how temperatures were maintained, were not provided. It was not stated whether the pH of the buffer solutions was ever measured after treatment, or whether the sterility of the test solutions was determined at any time during the study. It was not reported whether the test samples were stored prior to analysis.

2. A material balance could not be determined; quantitative data were provided only for benzyl diethyl. Volatiles were not trapped.

3. Transformation products were not quantified or identified. Sample chromatograms were not provided, so it could not be confirmed that benzyl diethyl was the only compound of significance in solution.

IV. REVIEWER'S COMMENTS

1. The study authors' reported the replicate data in terms of "mean peak height" (pp. 18, 20-22). Data in terms of concentration (g/L) and percent of applied were reported only as averages (pp. 19, 23).

2. A phosphate buffer was used at pH 5 and 7 (p. 11); acetate and borate buffers are preferred because of the potential for interference and interaction.

3. The experiments were conducted using unlabeled test material.

4. Physico-chemical properties of benzyl diethyl, including the solubility of benzyl diethyl in water, were not provided.

V. REFERENCES

1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 161-1. Hydrolysis studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.

Attachment 1: Structure of Test Material

Denatonium benzoate [Benzyl diethyl; Bitrex]

IUPAC Name:	Cyclohexylmethyl-[2- [(2,6-dimethylphenyl)amino]- 2-oxoethyl]-diethylammonium benzoate.
CAS Name:	Not reported.
CAS Number:	003734-33-6.
SMILES String:	c1ccccc1CN(CC)(CC)(OC(=O)c2ccccc2)CC(=O)Nc3c(C)cccc3C (EPI Suite, v3.12 SMILES).